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Experimental Studies on the Inhibitory Effect of Spleen Cells on the Growth of Transplanted Tumors

by

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INTRODUCTION

Since tumor antigens can be demonstrated by a new technique[13], immune responses to homografted, isografted, induced, and spontaneous tumors have recently become subjects of great interest.

Clinicians occasionally encounter a case of spontaneous regression of cancer. EVERSON and COLE[7] have collected 176 cases of spontaneous regression of cancer, and some examples of longterm arrest of the growth of cancer, or delayed recurrence or delayed metastasis of cancer. Such cases suggest the presence of biological control of the cancer. Many investigators consider that these phenomena are attributable to cell-bound antibodies. SOUTHAM[30] stated that the host defense mechanism is probably a cell-mediated type of immune response (lymphocytes) and not an antibody response.

Biological treatment aimed at the enhancement of non-specific host resistance has been studied for several years by various investigators. FICHERA (1934)[10] used extracts of spleen, thymus, and bone-marrow. VON THEILHAVER[41] first employed extracts of spleen, but later implanted portions of spleen from young animals. They observed some retardation of cancer growth, resorption, and even temporary disappearance of the cancer.

In the present study, the author attempted to immunize non tumor-bearing hosts ageinst transplantable tumor and to enhance host resistance in tumor-bearing animals by the implantation of the fresh spleen cells.

CHAPTER 1 CHANGES IN TRANSPLANTABILITY AT DIFFERENT SITES OF INOCULATION

MATERIALS AND METHODS

Male albino rabbits weighing about 2.0 kg were divided into 2 groups of 10 rabbits each. One group was injected intratesticularly with 1.0 ml of tumor suspension, and the other group was injected subcutaneously with the same volume. The tumor used was Brown-Pearce carcinoma which had been grown in the testicle and metastasized. After

the necrotic areas were removed, fresh tumor specimens were minced and suspended in isotonic salt solution at a concentration of 1.0 g per ml.

The incidence of "takes" was checked by autopsy 3 weeks after inoculation. The latent period of tumor growth was denoted to be the interval between transplantation and the first appearance of a palpable nodule.

RESULTS

Table 1 shows the differences in numbers of "takes" in the 2 groups (intratesticular route and subcutaneous route) ; these were analysed statistically.

Table 1 Numbers of "takes" in different inoculated site

Group	Numbers of takes	Numbers of non-takes	Total
Intratesticular	9 (7.5)	1 (2.5)	10
Subcutaneous	6 (7.5)	4 (2.5)	10
Total	15	5	20

( ) : theoretical frequency

The transplanted tumor grew in 90% of the animals inoculated by the intratesticular route, and in 60 % of those inoculated by the subcutaneous route. The difference in incidence of "takes" was calculated according to the x-square distribution.

where $x_S^2 = 3.100$,

ϕ (degree of freedom) $= 1$,

$x_{1\%}^2 = 6.635$,

$x_{5\%}^2 = 3.841$,

therefore, $x_S^2 < x_{5\%}^2$

The difference in incidence of "takes" in the 2 groups was not significant.

Table 2 Latent period of tumor growth in different inoculated site

Group	Latent period	Degree of freedom	Variance ratio
Intratesticular	5 8 10 11 12 12 14 14 13	8	2.262
Subcutaneous	8 8 10 12 12 12	5	

Table 2 summarizes the latent period of tumor growth and its statistical analysis. A test for the difference of the mean latent period was carried out in the distribution of F, with the formula

$$F^{n_1-1}_{n_2-1} = \frac{u_1^2}{u_2^2} \quad (u_1 > u_2)$$

$$u_1 = \frac{\Sigma (Xi - \bar{X})^2}{n_1 - 1} \qquad i = 1, 2, \cdots\cdots, n$$

and

$$u_2 = \frac{\Sigma (yi - \bar{y})^2}{n_2 - 1} \qquad i = 1, 2, \cdots\cdots, n$$

sample mean ; $\bar{X} = 11.0$, $\bar{y} = 10.3$, $u_1^2 = 8.750$, $u_2^2 = 3.868$,

$$\phi_1 = 8, \ \phi_2 = 5, \ Fs = 2.262, \ F_5^8(5\%) = 4.82,$$

$$Fs < F(5\%)$$

Therefore, the population variance was homogeneous. Next, the universal variation of 2 samples was calculated with the formula

$$\omega^2 = \frac{(n_1 - 1)u_1^2 + (n_2 - 1)u_2^2}{n_1 + n_2 - 2} = 6.872$$

$$\omega = 2.621$$

$$ts = \frac{\bar{X} - \bar{y}}{\omega} \sqrt{\frac{n_1 n_2}{n_1 + n_2}} = 0.867$$

Degree of freedom ; $\phi = 13$, $t(5\%) = 2.160$,

$$ts < t(5\%)$$

The difference was not significant.

Thus, the incidence of transplant "takes" and the latent periods did not differ significantly with the site of inoculation.

CHAPTER 2 INFLUENCE OF INTRAVENOUS INJECTION OF SPLEEN CELL SUSPENSION ON THE LATENT PERIOD OF TUMOR GROWTH

MATERIALS AND METHODS

Ten male albino rabbits weighing about 2.0 kg were injected intratesticularly with 1.0 ml of Brown-Pearce tumor suspension. Four or 5 days later, each animal was injected intravenously with 5 0 ml of a suspension of fresh spleen cells which had been collected under sterile conditions from normal adult rabbit.

Spleens were teased in isotonic salt solution forced through a fine-meshed stainless steel sieve with a flat-ended china pestle, washed twice, and resuspended in isotonic salt solution. The final volume of the suspension was adjusted to about 1.0 g of spleen tissue in a volume of 5.0 ml. In samples diluted with Tyrode's solution containing 0.05% trypan blue, unstained cells were registered as living.

The incidence of "takes" and the latent period of tumor growth were noted as described previously.

RESULTS

Table 3 shows the incidence of "takes" and the latent period of tumor growth as modified by the intravenous injection of homologous spleen cells. The results described in chapter 1 served as controls.

In this group, the incidence of "takes" was 70%. According to the χ-square distribution, the difference in the incidence of "takes" between animals treated with normal spleen cells and untreated controls was not statistically significant.

The curves of tumor "takes" are presented in Fig. 1, which shows that the latent period was longer than in the controls. The difference in the mean latent period was analyzed by F distribution.

Table 3 Influence of intravenous injection of spleen cells on the incidence of "takes" and latent period of tumor growth

Rabbit No.	Latent period (days)	Takes	Interval# (days)
1	22	+	4
2	22	+	4
3		−	4
4	20	+	5
5	15	+	5
6	9	+	4
7	9	+	4
8		−	5
9	13	+	4
10		−	4

Tumor was inoculated in the testicle

#: Between spleen cell injection and tumor inoculation

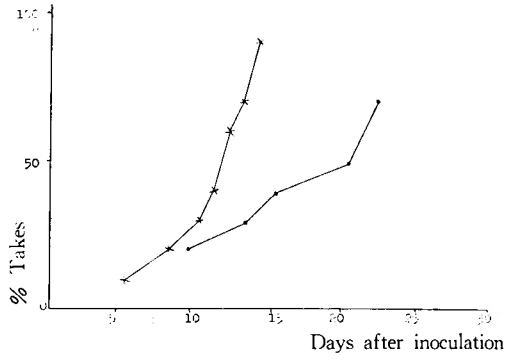

Fig. 1 Incidence of "tekes" and latent period or tumor growth

●——● : Group treated with homologous spleen

×——× Control group

The variance ratio=1.322, F(5 %)=4.15,

Therefore, the population variance was homogeneous.

$$ts=1.679,\ t(5\%)=2.145$$

The difference was not significant. Thus, the intravenous injection of spleen cell suspensions did not prolong the latent period of tumor growth.

CHAPTER 3 INFLUENCE OF INTRAVENOUS INJECTION OF SPLEEN CELL SUSPENSION ON TUMOR-BEARING ANIMALS

MATERIALS AND METHODS

Male albino rabbits weighing about 2.0 kg were divided into 2 groups of 10 rabbits each. In one group 2.0 ml of the tumor suspension was implanted into right testicle, and 5.0 ml of spleen cell suspensions was injected intravenously 8 days and 14 days later. The other group served as controls. The survival rates were noted.

RESULTS

Table 4 shows the survival time in days.

Table 4 Influence of intravenous injection of spleen cells on survival time

Group	Survival time (days)	Variance ratio
Treatment with spleen cells	19 22 16 21 17 20 20 22 19 24	3.78
Control #	20 10 16 10 19 23 23 15 14 14	

: Animal inoculated tumor suspension alone

Variance ratio=3.78,

$$3.18=F_9^9(5\%)<Fs<F_9^9(1\%)=5.35$$

The variance was not homogeneous.

$$ts = 2.16 < to = 2.228$$

The difference between mean survival times was not significant, and no significant prolongation of survival time was observed.

CHAPTER 4 EFFECT OF ADDING SUSPENSIONS OF AUTOLOGOUS SPLEEN CELLS TO TRANSPLANTABLE TUMOR CELL SUSPENSIONS IN NON-TUMOR-BEARING HOSTS

MATERIALS AND METHODS

The spleens were removed from male albino rabbits weighing about 2.0 kg, and the autologous spleen cells were harvested immediately in the usual manner.

A mixture of 1.0 g of tumor suspension and 1.0 g of autologous spleen cell suspension in 2.0 ml of isotonic saline solution was injected subcutaneously in the right inguinal area, and 1.0 g of tumor suspension alone was injected subcutaneously in the left inguinal area.

About 7 weeks later, 2.0 ml of isotonic saline solution containing 2.0 g of tumor cells was injected subcutaneously in the back.

"Takes" were ascertained as the appearance of induration by palpation. The incidence of "takes", the latent period, the fate of the tumor, and the histologic changes associated with admixture of autologous spleen cells were examined. Finally, the degree of regression was determined at autopsy. The results described previously in chapter 1 served as controls.

RESULTS

A. The incidence of "takes"

a) The difference between the two sides of the same animal

The difference in numbers of "takes" between the two sides were analyzed statistically as shown in Table 5.

Table 5 Influence of adding suspension of autologous spleen cells on "takes"

Side	Numbers of takes	Numbers of non-takes	Total
Right[a]	2 (4)	6 (4)	8
Left[b]	6 (4)	2 (4)	8
Total	8	8	16

a : Mixture of tumor cells suspension and autologous spleen cells suspension
b : Tumor cells suspension alone
( ) : Theoretical frequency

The transplanted tumor grew in 25% on the right side (mixture of tumor suspension and autologous spleen cell suspension).

However, it grew in 75% on the left side (tumor suspension alone). The difference in incidence of "takes" was calculated according to the x-square distribution.

where $x_0^2 = 4.00$, ϕ (degree of freedom) $= 1$,

$x_{1\%}^2 = 6.635$, $x_{5\%}^2 = 3.841$,

therefore, $x^2_{1\%} > x^2_S > x^2_{5\%}$

The difference in "takes" between the two sides was significant.

b) The difference in left side results between treated and control groups

The difference in the incidence of "takes" between a group treated with autologous

Table 6 Difference of numbers of "takes"

Group	Numbers of takes	Numbers of non-takes	Total
Treated with spleen cells	6 (5.3)	2 (2.7)	8
Control	6 (6.7)	4 (3.3)	10
Total	12	6	18

( ) : Theoretical frequency

spleen cells and a control group is shown in Table 6, and analyzed statistically according to x-square distribution.

where $x^2_S = 0.485$,

ϕ (degree of freedom) $= 1$,

$x^2_{5\%} = 3.841$, $x^2_{1\%} = 6.635$,

therefore, $x^2_S < x^2_{5\%}$

The difference in "takes" between the 2 groups was therefore not significant.

B. The latent period of tumor growth

Table 7 shows the statistical analysis of the latent period of tumor growth in the left side (tumor suspension alone). A test for the difference of the mean latent period was carried out in the F distribution.

Table 7 Influence of adding suspension of autologous spleen cell on latent period of tumor growth

Group	Latent period	Degree of Freedom	Variance
Treated with spleen cells	7 7 8 9 10 11	5	1.447
Control #	8 8 10 12 12 12 12		

Tumor was inoculated subcutaneously

: Described previously in Chapter 1

The ratio of variance was $1.447 < F(5\%) = 5.05$

Therefore, the population variance was homogeneous.

Then, $\omega^2 = 3.270$

$Fs = 2.651 < F^1_{10}(5\%) = 4.96$

The difference between mean latent period was not significant.

C. The fate of transplanted tumors

a) Primary challenge

In 6 rabbits, no tumor grew in the right inguinal area, and degeneration and necrosis were found at the site of inoculation within 7 to 17 days. Tumors developing in the left inguinal area regressed within 30 days after the absorption of the necrotic debris of the tumor tissue in these animals.

Two rabbits died within one month as a result of cachexia. In one of these 2 rabbits, although the tumor on the right side was necrotic, the tumor on the left side grew progressively. The results of the present experiments are summarized in Table 8.

Table 8 Fate of transplanted tumor

Animal No.	Inoculated side		Prognosis (days)
	Right (days)	Left (days)	
1	Necrosed (12)	Regressed (30)	Alive
2	Necrosed (13)	Regressed (25)	Alive
3	Necrosed (7)	Progressive	Died (26)
4	Necrosed (7)	Regressed (28)	Alive
5	Necrosed (7)	Non-takes	Alive
6	Necrosed (17)	Non-takes	Alive
7	Progressive	Progressive	Died (29)
8	Necrosed (8)	Regressed (22)	Died (42)#

: Rabbit died as a result of intercurrent intestinal infection with diarrhea but was negative for the presence of tumor at autopsy

b) Secondary challenge

All 5 rabbits in which the primary transplanted tumor regressed regained their normal weight and became healthy.

The subsequent challenge with the Brown-Pearce carcinoma in these same rabbits was unsuccessful. The rabbits were completely refractory to the growth of the Brown-Pearce carcinoma. None of the animals were found to have tumors at autopsy. Table 9 shows the number of "takes" after the secondary challenge.

Table 9 "Takes" of secondary challenge

Animal No.	Takes	Inoculated time# (days)	Sacrificed time# (days)
1	—	32	63
2	—	48	92
3	—	46	90
5	—	48	88
6	—	54	85

: After primary challenge

D. The histological findings

The tumor tissue used in the present experiment showed a typical medullary pattern microscopically, and the peripheral aspect of the tumor showed incomplete encapsulation and little inflammatory reaction.

When a suspension of autologous spleen cells was added to the tumor cell suspension, degeneration and necrosis were noted in all areas of the tumor. These areas become infiltrated with lymphocytes, granulocytes, and plasmacytes. Complete encapsulation with connective tissue was noted in this outer marginal area. However, tumor cells remained apparently viable at the periphery of the tumor at this period, and inflammatory cells surrounded these living tumor cells as shown in Fig. 2 and 3.

In these animals, necrosis of metastasis to the omentum were observed at autopsy as shown in Fig. 4. Microscopically, typical carcinomatous tissue was observed at the site of subcutaneous inoculation in the animals which died with metastases.

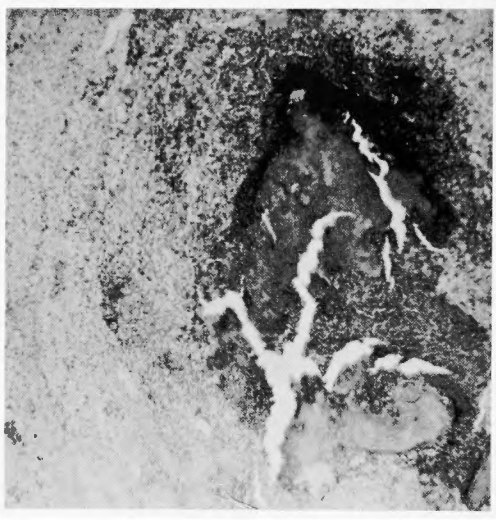

Fig. 2 Photomicrograph of transplanted tumor. Subcutaneous tumor. Degeneration and necrosis are noted. Hematoxyln and eosin stain. ×120.

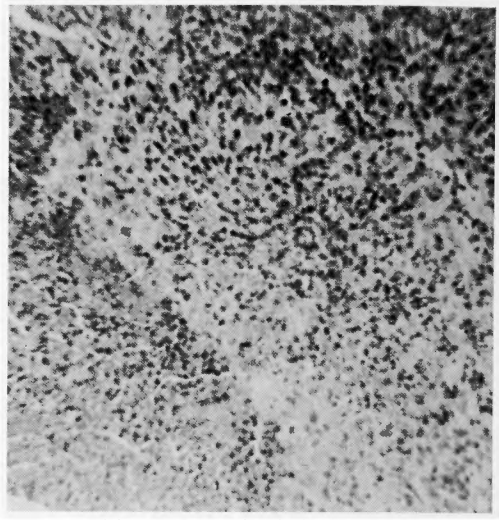

Fig. 3 Photomicrograph of the same preparation as Fig. 2. Inflammatory cells surround the living tumor cells. Hematoxylin-eosin stain. ×480.

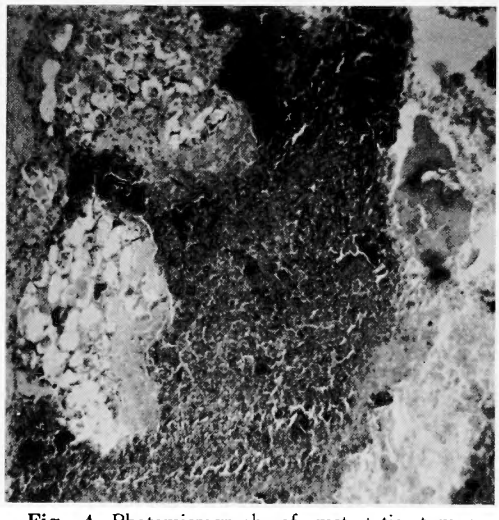

Fig. 4 Photomicrograph of metastatic tumor. Omentum. Necrosis are noted. Hematoxylin and eosin stain. ×120.

CHAMTER 5 INFLUENCE OF INOCULATION OF SUSPENSION OF SPLEEN CELLS MIXED WITH TUMOR CELLS IN TUMOR-BEARING HOST

A. Influence on survival time

a) Treatment with homologous spleen cells

MATERIALS AND METHODS

Male albino rabbits, weighing about 2.0 kg, were divided into 2 groups. Brown-Pearce carcinoma in a dose of 1.0 g was injected into each testicle. The tumor usually grew rapidly, metastasized within 8 to 12 days and produced bloody ascitic fluid.

In one group of animals, the testicular tumor was removed from one side 8 to 14 days later. Subsequently, a mixture of these tumor cells and homologous spleen cells was prepared as usual. Within 8 to 14 days after the primary inoculation, this mixture was in the inguinal region.

Another group served as controls. The survival times were noted.

RESULTS

Table 10 shows the survival time in days.

Table 10 Effect of homologous spleen cells on survival time

Group	Fate of mixture #	Survival time (days)					
Treatment with spleen cells	Absorbed	17	17	11	13	14	
	produced necrotic debris	21	27	16	19	30 over	
Control		14	13	14	14	14	13
		11	15	15	15	15	

: The mixture of Brown-Pearce carcinoma cells and homologous spleen cells

Variance ratio $=60>F_9^9(1\%)=5.34$

The population variance was not homogeneous.

In the test of difference in t-distribution,

$$ts=2.59> \quad to=2.262$$

The difference was significant at the 5% level.

In Fig. 5 the two groups are compared. A significant prolongation of life can thus be achieved by the addition of homologous spleen cells.

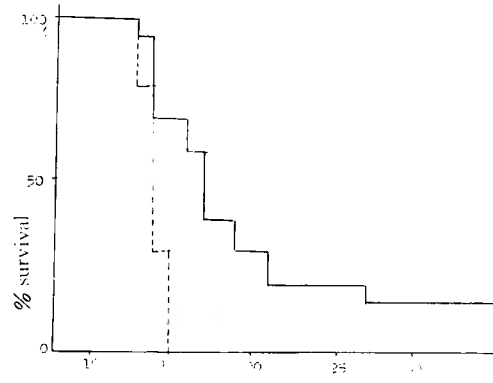

Fig. 5 Percentage survival of 2 groups
- - - : Group treated with homologous spleen cells
——— : Control group

Rabbits treated with homologous spleen cells were subdivided into 2 groups of 5 rabbits each.

Group 1 : The mixture of tumor cells and homologous spleen cells was absorbed without any growth of tumor.

Group 2 : The mixture produced necrotic debris within 7 to 8 days after inoculation.

The difference in the mean survival time was analyzed statistically.

The ratio of variance was 9.51, then

$$15.98=E_4^4(1\%)>Fs>F_4^4(5\%)=6.37$$

Therefore, the population variance was not homogeneous. In the test of difference in mean survival time in t-distribution

$$ts=2.804>to=2.776$$

The difference was significant at the 5% level, showing that the prolongation of life in group 2 was significant.

b) Treatment with autologous spleen cells

MATERIALS AND METHODS

Male albino rabbits weighing about 2.0 kg were used. The same method of implantation of tumor as described in section a) was employed in this experiment.

Within 7 to 10 days after inoculation, splenectomy was performed and suspensions of autologous spleen cells were harvested as usual. The tumor developing in one testicle was removed at the same time as the spleen. Then, a mixture of these tumor cells and autologous spleen cells was injected subcutaneously in the inguinal region, and the survival times were recorded.

RESULTS

Table 11 represents the survival time.

Table 11 Effect of autologous spleen cells on survival time

Group	Fate of mixture #	Survival time (days)					
Treatment with spleen cells	Absorbed	12	17	10	12	14	
	Produced necrotic debris	14	21	15			
Control		14	13	14	14	14	13
		14	15	15	15		

: The mixture of Brown-Pearce carcinoma cells and autologous spleen cells

Variance ratio $=21.666>F_9^7(1\%)=5.62$

The population variance was not homogeneous. A test for the difference of the mean survival time was carried out in the t-distribution.

$$ts=0.219<to=2.36$$

Therefore, the difference was not significant.

Fig. 6 shows the survival rates of the 2 groups.

Next, rabbits treated with autologous spleen cells were subdivided into 2 groups.

Group 1 : In 5 rabbits, the mixture of tumor cells and autologous spleen cells was absorbed without any growth of tumor.

Group 2 : In 3 rabbits, the mixture produced necrotic debris within 6 to 8 days after inoculation.

The difference in the mean survival time between the 2 groups was calculated in the F-distribution.

Variance ratio $=2.048<F_4^2(5\%)=6.94$

The population variance was homogeneous.

$$Fs=2.57<F_6^1(5\%)=5.99$$

Therefore, the difference was not significant.

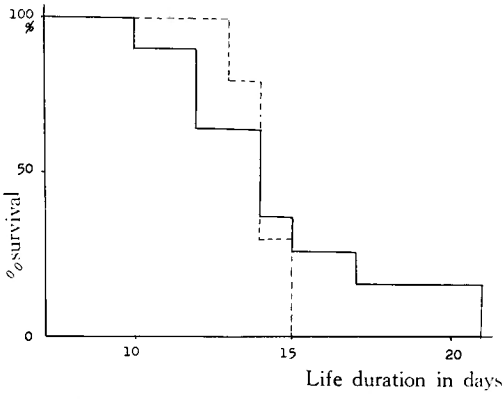

Fig. 6 Percentage survival of 2 groups
— — : Group treated with autologous spleen cells
- - - : Control group

B. Quantitative estimation of the phagocytic activity of the reticuloendothelial system

MATERIALS AND METHODS

The animals used in this study were the same rabbits as in section A.

The rate of disappearance from the circulation of congo-red (1 ml/kg body weight of a 1 % congo-red solution) injected intravenously was measured by a modification of the method of ADLER and REIMANN. Clearance of congo-red (Merk 1339, Grüber) from the blood proceeds at a constant rate so that the congo-red index K is equal to

$$\frac{Cb}{Ca} \times 100$$

where Ca=concentration 4 minutes after injection, and Cb=concentration 60 minutes after injection.

The K value in normal rabbits is 40～60% (YAMAGATA)[44].

RESULTS

Fig. 7 illustrates the value of K before the intratesticular implantation of Brown-Pearce carcinoma, and about 7 days after the inoculation of a mixture of tumor cells and spleen cells.

In the group in which survival time was prolonged, 6 out of 8 animals (75%) showed a rise in the phagocytic activity of the reticuloendothelial system after the inoculation of this mixture.

During the course of this experiment, the K values were calculated before inoculation of the tumor, at the same time as implantation of the mixture of tumor cells and spleen cells, and thereafter once each week. The K values are summarized in Table 12.

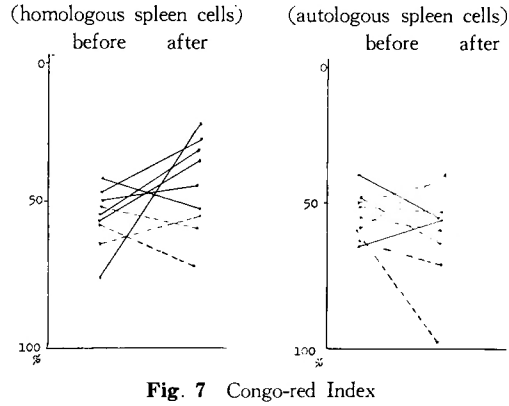

Fig. 7 Congo-red Index

Table 12 Values of K in the course of experiment

Group	Animal No.	Values of K (%)				Survival time (days)
		a	b	c	d	
Treatment with homologous spleen cells	1	64.7	52.8	54.9		21
	2	45.3	39.9	40.9	79.4	27
	3	49.5	50.9	42.7	36.3	30 over
	4	47.7	51.0	27.1		16
	5	54.1	49.6	20.2		19
Treatment with autologous spleen cells	1	45.4	42.8	62.9		17
	2	58.8	32.1	98.3		14
	3	61.6	42.3	53.2	88.9	21
	4	47.0	26.4	39.2		15

a : Before inoculation of tumor
b : At the same time as implantation of the mixture
c : 7 days after implantation of the mixture
d : 14 days after implantation of the mixture

The phagocytic activity of the reticuloendothelial system in tumor bearing hosts rose after the injection of the mixture of tumor cells and spleen cells, and declined immediately before death.

C. Histological findings

In animals treated with homologous spleen cells, the residual tumor in the testicle showed complete encapsulation by connective tissue. The metastatic growths on the omentum showed that the tumor cells were surrounded by dense infiltration of lymphoid cells and revealed fragmentation or swelling of the nuclei as shown in Fig. 8. On the contrary, the animals treated with autologous spleen cells (after splenectomy) showed a thin infilt-

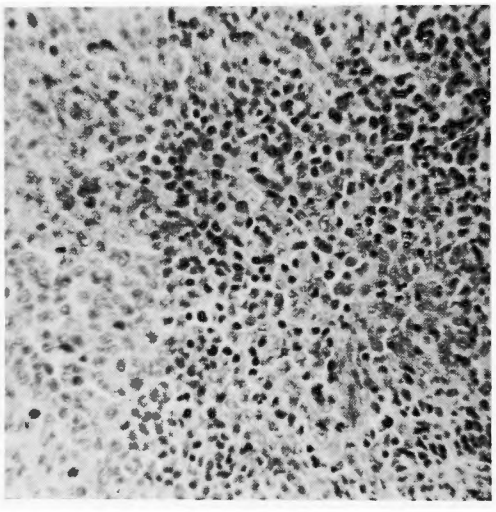

Fig. 8 Photomicrograph of metastatic tumor on the omentum. Animal treated with homologous spleen cells. Hematoxylin and eosin stain. ×480.

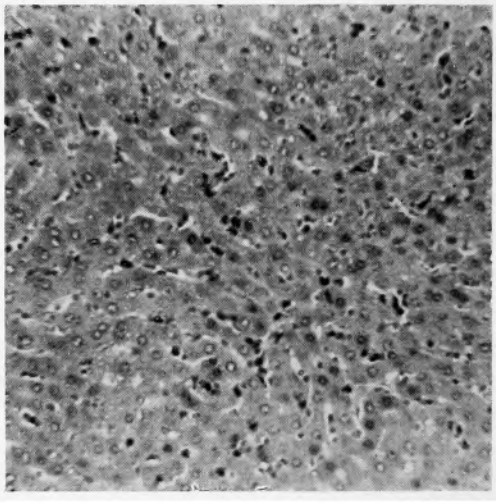

Fig. 9 Photomicrograph of liver in animal treated with homologous spleen cells. Hematoxylin and eosin stain. ×480.

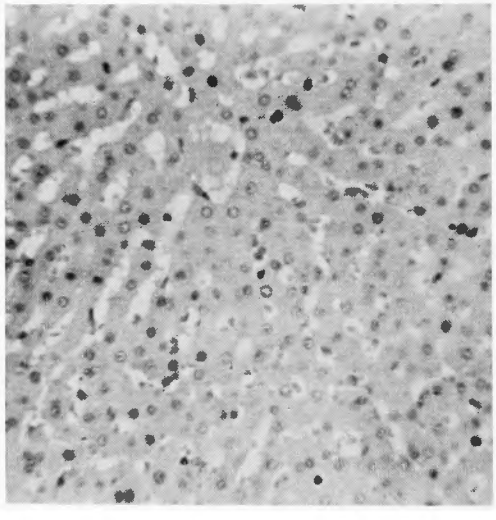

Fig. 10 Photomicrograph of liver in animal treated with autologous spleen cells. Hematoxylin and eosin stain. ×480.

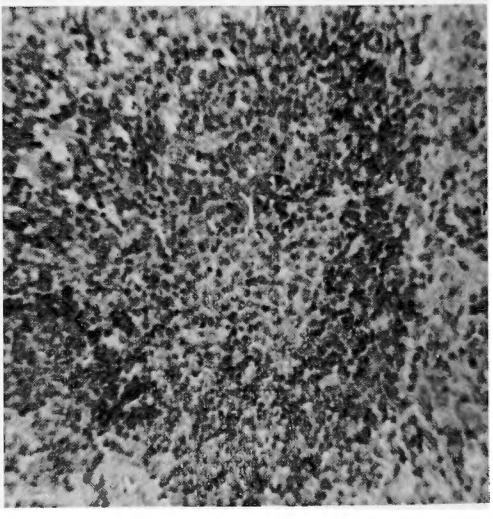

Fig. 11 Photomicrograph of spleen in animal treated with homologous spleen cells. Hematoxylin and eosin stain. ×480.

ration of inflammatory cells.

In the rabbits treated with homologous spleen cells, the liver showed a considerable increase in the number of Kupffer cells, as shown in Fig. 9. No increase in the number of Kupffer cells was observed in the rabbits treated with autologous spleen cells as shown in Fig. 10.

The spleens of animals treated with homologous spleen cells revealed hyperplasia of follicles with proliferation of lymphocytic germinal centers as shown in Fig. 11.

DISCUSSION

The peculiarities of each tumor form a very important problem in experimental studies of animal tumors. Some investigators have doubted the usefulness of Brown-Pearce carcinoma in experimental studies because complete regressions occasionally occur spontaneously.

However, once metastases have appeared, spontaneous regression does not occur and the tumor grows and metastasizes like human carcinoma. BROWN and PEARCE[4] reported that transplanted tumors grew in 100% of animals inoculated by the intratesticular route, and in only 20 to 25% of animals inoculated by the subcutaneous route.

The author used a comparatively large quantity of fresh tumor specimens acquired from metastatic lesions. As a result, the number of "takes" in subcutaneous inoculation was increased to 60%. The difference in incidence of "takes" between subcutaneous and intratesticular inoculations was not significant statistically. Therefore, the author selected either the intratesticular or the subcutaneous route according to the design of the experiment.

The conception that the reticuloendothelial system, especially the spleen, is concerned essentially in host resistance to cancer has been supported by many investigators, because no primary cancer develops in the spleen and secondary cancer only very rarely. DOMAGK, ILFELD, BAUER, DECKNER and KALLOS[8] reported that metastatic growths developed in only 1 or 2 of a large series of rabbits implanted with Brown-Pearce carciaoma.

H. APOLANT (1911)[1] reported that host resistance to the proliferation of transplanted neoplasma was reduced after splenectomy, and OJIMA[29] reported that splenectomy potentiated carcinogenesis, proliferation of tumors and metastasis in experimental animals. In view of these findings, BRAUNSTEIN[5] studied more widely the inhibitory effect of the spleen on the proliferation of cancer.

In the author's experiment, the intravenous injection of living homologous spleen cells after tumor implantation caused a slight prolongation of the latent period and a slight decrease in the number of "takes", but these differences were not significant statistically. In the hosts in which tumors had already developed, the survival rate was not increased by repeated intravenous injections of homologous spleen cells. The author concludes that the intravenously injected homologous spleen cells are probably caught in the recipient's reticuloendothelial system, causing a temporary increase of non-specific resistance. In the hosts with already developed tumors, however, this injection of spleen cells has no effect because of the decline of reticuloendothelial function. AKASU et al.[2] reported that the tumor-bearing host's reticuloendothelial function and lymphocyte count decreased together as cancer progressed. SUENAGA[31] found that reticuloendothelial function increased 4 to 6 days after tumor implantation, and decreased progressively thereafter.

In the next experiment, the author inoculated a mixture of autologous spleen cells and

tumor cells for the purpose of estimating the inhibitory effect of spleen cells on the growth of transplanted tumor. This mixture produced necrotic debris 1 to 2 weeks after inoculation. The transplanted tumor took and grew on the left side (inoculated with tumor alone) as well as in the control group, but showed regression and absorption of necrotic debris on the right side (inoculated with the mixture).

A subsequent challenge with Brown-Pearce carcinoma in these same rabbits was unsuccessful. The rabbits were completely refractory to the growth of Brown-Pearce carcinoma and appeared to have acquired specific resistance to this particular tumor. Necrotic tumor tissue may have served as an antigen, stimulating a specific antibody response to the tumor from which the antigen was derived. The influence of allogeneic inhibition was probably negligible in this experiment.

STRAUSS et al[32-36]. reported that host resistance was increased by the absorption of electrocoagulated antigenic material in clinical and experimental investigations. On the other hand, in spite of the production of necrotic debris, no regression of tumors was observed in the hosts with already developed tumors which were inoculated with autologous spleen cells. The results of this experiment indicate that normal spleen cells inhibit the growth of tumors, and suggest that immunologic resistance to carcinoma in splenectomized hosts is afforded by other reticuloendothelial organs than the spleen in normal animals, but not in tumor-bearing animals since their reticuloendothelial function is depressed.

Survival time was prolonged in non-splenectomized hosts with already developed tumors by the inoculation of homologous spleen cells. This effect was especially obvious in the animals in which necrotic debris was present in the tumor. In these cases, the reticuloendothelial function obviously rose after the inoculation of the mixture of cancer cells and spleen cells, and histologic examination of the residual tumor and metastatic tumors revealed infiltration of inflammatory cells around the tumor cells and connective tissue encapsulation. These findings point to an increase in host resistance to cancer.

Many Investigators are of the opinion that reticuloendothelial function in tumor-bearing hosts is closely related to the course of the tumor, and that a mesenchymal reaction in the tumor tissue indicates a favorable prognosis[7)14)19)20)25)28)37)42)45)].

On the basis, however, of the decrease of delayed hypersensitivity and lose of humoral responses to antigen stimulation (SOLOWEY)[38)], others have described the tumor-bearing host as in a state of immune-tolerance or immune paralysis (HADDOW[13)], ISHIBASHI[18)]).

The main difficulty in the immune therapy of cancer is attributed to the dissolution of immune tolerance. Various materials have been used in an attempt to counteract immune paralysis, such as large amounts of gamma-globulin (HIRAI)[15)], infiltrating cells in the abdominal cavity (SUENAGA)[31)], autolysing tumor tissue of autochthonous tumor (ONO)[27)], spleen homogenate (NODA[26)] MIZUMOTO[24)]), nitrogen-mustard-treated cancer tissue (ASAKUMA)[3)], normal non-sensitized lymphocytes and leucocytes (BRUNSCHWIG[6)], TANAKA[40)], WOODRUFF[46)]), lymph node cells (SJÖGREN)[39)], etc.. These trials, have not yet achieved any significant clinical effect.

On the one hand, many investigations have been reported in which chemotherapy and radiotherapy of cancer inhibited host resistance, and increased the incidence of metastases of experimental tumors[11)16)17)21)].

On the other hand, attention has been paid to immunosupportive maneuvers and stimu-

lation of reticuloenothelial function. Further studies of this contradictory problem are necessary.

The results of the present study have led the author to conclude that spleen cells play an important role in enhancing host resistance to cancer, and that homologous spleen cells which have been sensitized to autologous tumor cells may be more effective than normal homologous or autologous non-sensitized spleen cells. KOLDOVSKY[23] suggests the possibility of specifically sensitizing in vitro the patient's own immune cells against his cancer to damage malignant target cells. KUDO[22] reported that the transplantation of tumor cells mixed with tumor- or BCG-sensitized spleen cells showed the best results.

It is most important that these transfer cells are injected as early as possible while reticuloendothelial function is still active.

SUMMARY

The author inoculated Brown-Pearce carcinoma intratesticularly or subcutaneously in rabbits, and then treated them with a mixture of homologous or autologous normal fresh spleen cells and tumor cells in order to heighten the host resistance to cancer. The following conclusions were drawn :

1) The intravenous injection of autologous or homologous normal spleen cells had no effect in normal or tumor-bearing animals.

2) Non-tumor-bearing animals injected subcutaneously with a mixture of tumor cells and autologous spleen cells acquired specific immunity to canncer cells. The absorption of necrotic debris of tumor tissue was noted to be a necessary condition.

3) In tumor-bearing animals, this mixture of autologous spleen cells was not effective.

4) Tumor-bearing animals treated with a mixture of tumor cells and homologous spleen cells showed a significant prolongation of survival time. This effect was more obvious in those with necrotic debris in their tumor tissue.

5) The congo-red index increased after the inoculation of this mixture in the group with a prolonged survival time.

6) Histologic examination showed augmentation of mesenchymal reaction in the tumor tissue, hyperplasia of lymphoid germinal centers in the host's spleen, and an increased number of Kupffer cells in the host's liver in the group with a prolonged survival time.

These reaction were not observed in those in which the survival time was not prolonged. A mixture of tumor cells and normal fresh spleen cells was found to reinforce host resistance to cancer.

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和文抄録

脾細胞の移植癌生育抑制効果に関する実験的研究

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癌の自然退縮が時に臨床的に認められる事は癌に対する生物学的制御機構が働いている事を暗示するが，最近癌特異抗原の存在が証明されるに至り，腫瘍免疫の概念が再び脚光をあびる様になつた．

脾臓には原発腫瘍や転移腫瘍の発生が極めて稀である事から，担癌体の防衛機構における脾の役割を強調する研究は古くから多数報告されている．又，この防衛機構では細胞抗体が主役を演じ，リンパ系細胞が主体である事が一般に認められている．本研究では兎のBrown-Pearce癌を用いて，正常自家或は同種脾細胞を投与し宿主の抵抗性を増強させる事を試みた．

最初にBrown-Pearce癌細胞1.0gを含む生理食塩水浮遊液を睾丸内と皮下に移植して生着率及び腫瘤形成迄の潜伏期間を検討した処，両者の間に有意の差を認めなかつたので，観察の便利な様に両者を適宜に選んだ．実験結果は次の如く要約される．

1）正常家兎に癌細胞接種後4乃至5日目に同種脾細胞浮遊液（約1gの脾より調製）を静脈内注射を行なつたが，腫瘍生育抑制効果は得られなかつた．又，担癌家兎に同様の処置を行なつたが延命効果はみられなかつた．

2）正常家兎の一側鼠蹊部皮下に腫瘍一自家脾細胞混合浮遊液を接種し，反対側鼠蹊部皮下に腫瘍のみを移植した処，混合液接種部に7～17日目頃腫瘍組織壊死物質を産生し，これが吸収されるにつれて反対側の腫瘍が約4週間以内に退縮していき，宿主はBrown-Pearce癌に対して特異的免疫を獲得し，約7週間後に最初の倍量の腫瘍細胞を移植したが全例生着しなかつた．

3）担癌家兎に同様の方法で実験を行なつたが延命効果は認められなかつた．この事から担癌体の抵抗性強化には宿主の脾が重要な役割を演ずるものと推測される．

4）家兎の両側睾丸内に同量のBrown-Pearce癌を移植し，8乃至14日後に一側の睾丸腫瘍を摘出し，この腫瘍細胞と同種脾細胞との混合浮遊液接種を鼠蹊部皮下に行なつた．この混合浮遊液接種部位に組織壊死物質を産生し，吸収された群では有意の延命効果を認めたが，壊死物質を産生することなく，接種後間もなく吸収された群では延命効果は著明でなかつた．

5）担癌家兎の網内系機能を腫瘍移植前，脾細胞接種時，その後7日間隔で測定した．測定はAdler-Reimann氏変法によるコンゴー赤係数を用いた．延命効果を認めた群では脾細胞接種後明らかに網内系機能は上昇したが，死亡直前には急激に低下した．

6）組織学的検査によると延命効果のあつた担癌家兎では腫瘍辺縁の間質反応の増強，脾のリンパ胚中心の増殖，肝のKupffre細胞の増加等が認められたが，延命効果を認めなかつた群ではこの様な組織反応を認めなかつた．

以上の所見から，正常脾細胞には明らかに癌生育抑制効果を認めるが，更に種々の条件附けによつて（標的腫瘍細胞に対する感作等）臨床的応用の可能性を期待出来ると考えられる．